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Mouth Exposure to Carbohydrate Prior to Exercise Possibly Impairs
the Efficacy of Carbohydrate Mouth Rinsing during Exercise
Kevin Decker

A thesis submitted to the Graduate Faculty of

JAMES MADISON UNIVERSITY

In

Partial Fulfillment of the Requirements

for the degree, of

Master of Science

Department of Kinesiology

May 2017

FACULTY COMMITTEE:

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Dr. Nicholas D. Luden

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Dedication Page

I would like to dedicate my thesis to family and friends for all the love and support throughout the process. I am blessed to have you all in my life as I graduate with a M.S. in Exercise Physiology from James Madison University and begin to pursue a Ph.D. in Rehabilitation and Movement Science with a concentration in Exercise Physiology at Virginia Commonwealth University.

Acknowledgements

I would first like to acknowledge Dr. Michael Saunders for his guidance throughout my years at James Madison University. He has taught academic success as my professor, shared invaluable wisdom as my mentor, and continuously provided me feedback and support as my Committee Chair. It is a clear inference that you have been ‘very likely’ beneficial in my life.

I would like to acknowledge Dr. Nick Luden and Dr. Chris Womack as members of my thesis committee members and professors. More specially, I would like thank Nick for helping me develop my critical thinking skills within the research by asking even more questions than before. I would like to thank Chris for making me see the big picture not only with my research but also with my professional goals.

To my research partner, Nikolai Hladick, thank you for a collaborative effort and making the entire thesis process enjoyable. In addition, I would like to thank the research assistants; Kendall Clark, Megan Pedersen, Joshua Forrest, Erin McConnell, Nick Valle, and Angelique DeMeo for all their hard work and early mornings.

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Table of Contents

Dedication Page.....	ii
Acknowledgements.....	iii
Table of Contents.....	iv
List of Tables.....	v
List of Figures.....	vi
Abstract.....	vii-viii
I. Introduction.....	1-6
II. Methods.....	7-12
III. Manuscript.....	13-35
IV. Appendices.....	36-53
V. Reference.....	54-60

List of Tables

Table 1. Treatment Conditions for each experimental trial.....	7 & 16
Table 2. Physiological Responses during Cycling at 70% W_{\max}	22
Table 3. Physiological Responses during Time-Trial Cycling.....	23

List of Figures

Figure 1. Diagram of experimental procedure.....	10 & 19
Figure 2. Treatment Effects on 30-km Time-Trial Performance.....	24

Abstract

Decker K. P., M. J. Saunders, N. D. Luden, C. J. Womack, and N. J. Hladick. Mouth Exposure to Carbohydrate Prior to Exercise Possibly Impairs the Efficacy of Carbohydrate Mouth Rinsing during Exercise. **Purpose:** Carbohydrate mouth-rinsing (CHO-MR) during intense endurance exercise has been associated with improved cycling performance, due to neurological influences. However, prior studies have reported the efficacy of CHO-MR is attenuated following a pre-exercise meal. To determine if this outcome is related to desensitization of CHO receptors (rather than metabolic effects following digestion), this study will investigate whether CHO-MR prior to exercise influences cycling performance when CHO-MR is also used during exercise. **Methods:** Eight trained cyclists (age, 24 ± 6 yr; height, 176 ± 6 cm; weight 75 ± 12 kg; VO_{2max} , 61 ± 8 ml/kg/min) completed three exercise trials, each consisting of 15-min of incremental, constant-load exercise followed by a simulated 30-km time-trial (TT). Treatment beverages in the trials were randomly counterbalanced: a) PL_PL: placebo before and during exercise, b) PL_CHO: placebo pre-exercise, CHO-MR during exercise, and c) R_CHO: CHO-MR before and during exercise. Physiological responses (VO_2 , V_E , RER, RPE, heart rate, blood glucose and lactate) were assessed during constant-load exercise and during the TT. Magnitude-based qualitative inferences were used to evaluate differences in responses between treatments. **Results:** TT performance was ‘possibly’ impaired (59% likelihood) with R_CHO (57.3 ± 3.6) versus PL_CHO (56.9 ± 3.0 min). Both trials were ‘likely’ slower than PL_PL (55.8 ± 3.1 min), but the reliability of performance data from this trial may have been impacted by measurement error, which limited our ability to determine the influence of CHO-MR during exercise. Physiological responses between treatments during constant-load cycling, and the TT were generally similar between all treatments. **Conclusion:** A pre-exercise CHO-MR had a possibly negative impact on cycling performance that also included CHO-MR during exercise. Although further evidence is required to validate this finding, our data suggests that desensitization of CHO receptors related

to recent CHO exposure may be partially responsible for previous reports that the efficacy of CHO-MR during exercise are attenuated by pre-exercise feedings. **Keywords:** CYCLING, CARBOHYDRATE, MOUTH-RINSING, PERFORMANCE, ERGOGENIC-AIDS.

Chapter I

Introduction

The influence of carbohydrate ingestion on endurance performance has been studied extensively. Muscle and liver glycogen are important fuel sources during prolonged endurance events (≥ 90 minutes) (10, 32), and athletes are generally instructed to consume high carbohydrate meals the night *before* and/or within a few hours prior to prolonged endurance events (31, 38, 46, 65), in order to insure that muscle/liver glycogen stores are high prior to exercise (16, 28). In addition, carbohydrate ingestion *during* prolonged endurance events is associated with improved performance (17, 21, 63), particularly when endogenous carbohydrate is limited. Carbohydrate replacement *during* exercise maintains higher rates of carbohydrate oxidation (65), thus making more energy available in the later stages of prolonged exercise (17, 37).

To maximize the ergogenic effects of carbohydrates, researchers have investigated the combined effects of carbohydrate feeding *before* and *during* endurance exercise. Wright et al. found cycling time to exhaustion was improved by 18% with pre-exercise feeding, and by 32% with during exercise feeding. However, a combination of pre- and during exercise carbohydrate feedings improved performance by 44%. Collectively, carbohydrate feedings before and during prolonged endurance exercise are believed to be ergogenic because they support higher rates of carbohydrate oxidation (65), maintain adequate blood glucose levels, and spare endogenous glycogen stores throughout exercise (10, 16, 18, 32).

More recently, the benefits of carbohydrate feedings *before* and *during* exercise have been observed in relatively short, high intensity (~ 60 minutes, $\geq 75\% \text{VO}_{2\text{max}}$) aerobic exercise (2, 9, 22, 36, 44), although not all studies report benefits (19, 43, 47). Jeukendup and colleagues found consumption of a 7.6% carbohydrate-electrolyte solution before and during a 40-km time trial improved performance by approximately one minute (2.3%). However, only 15 grams of exogenous carbohydrate were oxidized during the 60 min time-trial, while total energy expenditure was ~ 20 kcal/min (36). Similarly, McConnell and colleagues (43) reported that only

26% of carbohydrate ingested during a one-hour high-intensity exercise trial appeared in the peripheral circulation (22 of 84 grams). Although blood glucose levels were significantly increased by exogenous glucose ingestion, there was little to no effect on total carbohydrate oxidation under these exercise conditions (43). In addition, intravenous glucose infusion (at one g/min) had no effect on performance during a 40-km cycling time trial (13). Therefore, carbohydrate ingestion may be beneficial during high intensity aerobic exercise of ~ one hour, these effects cannot be explained by metabolic mechanisms (39).

Carter and colleagues were the first to investigate the effects of carbohydrate mouth rinsing on endurance performance. Rinsing the mouth with 25 ml of a 6.4% maltodextrin solution periodically during exercise (without ingestion) improved performance in a one-hour cycling time-trial by 3% versus a placebo mouth rinse (12). In a review by de Ataide e Silva and colleagues, at least nine studies replicated the enhancements in performance (ranging from 1.5% to 11.6%) using a carbohydrate mouth rinse protocol of similar intensity and duration (5). For athletes sensitive to incidences of gastrointestinal problems with carbohydrate ingestion during exercise may find carbohydrate mouth rinsing an alternative strategy (45, 48). Collectively, carbohydrate ingestion and mouth rinses during short, high intensity aerobic exercise has been shown to enhance performance (2, 9, 12, 14, 22, 36, 44, 50, 52–54), yet not all studies report these performance enhancements (1, 8, 34, 61, 62).

The ergogenic effects of carbohydrate mouth rinsing during exercise are believed to be related to neurological influences of carbohydrate sensed in the mouth. The mouth has oral-pharyngeal receptors that respond to taste, temperature, texture and nutrients sensed by gustatory neurons, which sends sensory information to the central nervous system for integration (4, 11, 40, 56). Stimulation of these receptors from carbohydrates in the mouth can induce dopaminergic pathways in the brain rewards center (3). For example, the tasting of sucrose elicited stimulation of dopamine regions in the brain even when a tube prevented digestion to the stomach (30). Chambers and colleagues used functional magnet resonance imaging (fMRI) to examine brain

responses to glucose, a sweet carbohydrate, and maltodextrin, a tasteless non-sweet carbohydrate. The fMRI revealed exposure of carbohydrates in the mouth, regardless of sweetness, activated the insula/frontal operculum, orbitofrontal cortex and striatum of the brain relating to the pleasure and reward (15). Frank and colleagues used fMRI to examine the effects of sucrose versus sucralose, a taste matched non-caloric sweetener, on brain activity. Sucrose elicited a greater response in the anterior insula, frontal operculum, striatum and anterior cingulate cortex than sucralose. Sucrose, but not sucralose stimulated dopaminergic midbrain areas, suggesting energy content rather than sweetness stimulates the central nervous system (25). Gant and colleagues used transcranial magnetic stimulation of the primary motor cortex to examine the effects of a non-sweet carbohydrate on corticomotor excitability and voluntary force production during 30 minutes of isometric elbow flexion. They demonstrated that the presence of carbohydrates in the oral cavity immediately increased corticomotor output and maximal voluntary force production (27). Bastos-Silva and colleagues reported carbohydrate mouth rinsing during exercise maintained electromyography activity of the quadriceps (6). This enhanced performance lasting at least 60 minutes, but not during supramaximal exercise (~ three minutes), indicating the role carbohydrate mouth rinsing has with alleviating central fatigue (59). In summary, enhanced performance from carbohydrate mouth rinsing is believed to be due to its actions on the central nervous system, stimulating the motor cortex and dopaminergic pathways, subsequently causing increased voluntary force production (27), reduced neuromuscular fatigue (6, 35), reductions in perceived exertion, and maintained motivational drive (11, 23).

The magnitude of the ergogenic effects of carbohydrate mouth rinsing on endurance performance can be influenced by the duration of mouth rinsing, concentration of carbohydrate solution, and total number of mouth rinses. Most studies rinse for five seconds (8, 12, 26, 49, 50, 52, 54, 55), but others have utilized a ten second rinsing protocol (15, 42). Sinclair et al. found both five and ten second mouth rinse protocols improve 30-minute cycling performance compared to placebo (57). Gam et al. reported that additional time spent mouth rinsing during

intense exercise is detrimental to performance because of disturbances in ventilation (26). The effects of different concentrations of maltodextrin mouth rinses were examined in two studies; both reported no difference between 3%, 6%, or 9% solutions (41), or between 6% or 16% solution on cycling performance (20). Most exercise research use a carbohydrate mouth rinsing protocol of relatively high mouth rinsing frequencies of eight or more times over ~ one-hour (12, 15, 23, 42, 50, 62), although some studies have used less (52, 55, 57). However, de Ataide e Silva and colleagues reported that no studies have directly investigated how mouth rinsing frequency influences performance outcomes (5), so further research is warranted in that regard.

Most studies that have reported ergogenic effects from carbohydrate ingestion/rinsing during exercise durations of ~ one-hour have testing subjects after an overnight fast (for purposes of nutritional standardization). Beelen and colleagues examined the effects of carbohydrate mouth rinsing after a pre-exercise meal, and observed no improvements in cycling performance (8). The authors speculated that pre-exercise feedings may attenuate the ergogenic effects of carbohydrate mouth rinsing during subsequent exercise, which would reduce the practical significance of this strategy (since pre-exercise feedings are recommended for reasons already discussed) (8). Lane and colleagues directly examined the impact of pre-exercise nutritional status on the effectiveness of carbohydrate mouth rinsing (42). Subjects were either fasted or fed a 2.5g/kg meal two hours prior to a 60-minute cycling time-trial utilizing a carbohydrate mouth rinses. Carbohydrate mouth rinsing during exercise improved mean power output by 3.4% after an overnight fast, but only 1.8% in a carbohydrate fed state. Although the efficacy of the carbohydrate mouth rinse was reduced in a fed state, the best mean performance time was reported in the trial which included pre-exercise carbohydrate feeding and mouth rinse during exercise (42). In a similar study, subjects were either fasted or fed a carbohydrate rich breakfast three hours prior to cycling to exhaustion at 60% W_{\max} utilizing a carbohydrate mouth rinse protocol. A maltodextrin mouth rinse solution improved time to exhaustion by 5.6 minutes after

an overnight fast and by 1.9 minutes in the fed state (23). The carbohydrate mouth rinse was thought to exhibit a more pronounced signal to the CNS during a physiological state of hunger.

The aforementioned outcomes may indeed be related to altered neural responses in a state of hunger (29). Van Rijn et al. measured neural responses using fMRI when the oral cavity was exposed to carbohydrates or artificial sweetener in a fasted and fed state. Carbohydrates activated more areas of the brain in state of hunger than satiety than the artificial sweetener (51), again suggesting the importance of energy over taste (25). In addition, neuronal activity in the hypothalamus is reduced following glucose ingestion, with greater reduction from 75 grams compared to 25 grams suggesting a dose-dependent response (58). Although brain activation appears to be blunted after carbohydrate feedings, the mechanisms responsible for the attenuation in performance using a carbohydrate mouth rinse during exercise are not fully understood. Therefore, the purpose of this study was to investigate if pre-exercise carbohydrate mouth rinses influence the efficacy of carbohydrate mouth rinsing during a 30-km performance cycling time-trial. This will help determine if the attenuated response is related to neurological influences related to desensitization of (or down-regulation of feedback from) oral-pharyngeal CHO receptors, as opposed to altered metabolic responses following digestion (i.e. such as changes in blood glucose, insulin, ghrelin, etc.). It was hypothesized pre-exercise carbohydrate mouth rinses will attenuate the neurological aspects of carbohydrate mouth rinsing during exercise, resulting in impaired 30-km cycling performance compared to no pre-exercise carbohydrate mouth exposure.

Assumptions, Limitations, Delimitations

During this study, it will be assumed that subjects are giving maximal efforts during all performance trials. The researchers will also assume that subjects adhere to pre-exercise behavioral and dietary protocols and instructions, in addition to adhering to all experimental protocols during trials. Accuracy of measurement instruments and competency of all researchers and assistants involved will be assumed. Due to homogeneity of the subject group, the results of

this study can only be applied to similarly trained subjects, between the ages of 18 and 45 years old. Trials will be performed on cycle ergometers in an exercise laboratory; as such, the practical application of the findings may be limited when applying the same feeding strategies in real-world competitions.

Chapter II

Methods

Subjects

Eleven trained cyclists were recruited from James Madison University and the surrounding area in Harrisonburg, Virginia. Subjects met the following inclusion criteria: 18 - 45 years of age, ≥ 2 years of experience in endurance cycling events, $VO_{2max} \geq 50$ ml/kg/min, consistent training prior to this study of ≥ 3 days/week, and ≥ 4 training sessions ≥ 2 hours in duration over the past 2 months. Subjects were classified as low-risk for health complications according to ACSM guidelines (60), and provided written informed consent prior to starting the study (appendix A). Experimental procedures were approved by the James Madison University Institutional Review Board (IRB #17-0084).

Study Design

The study utilized a double-blind placebo-controlled crossover design. Subjects who meet the inclusion criteria performed a maximal oxygen uptake (VO_{2max}) test, a familiarization trial and three experimental trials. Treatments utilized during the experimental trials are shown in Table 1. Trial order was randomly counterbalanced across subjects and separated by ≥ 7 days each. Subjects reported to the Human Performance Laboratory at James Madison University for a total of five visits within a six-week timeline.

Table 1: Treatment Conditions for each experimental trial. (PL = Placebo; CHO = Carbohydrate)

Treatment	Pre-exercise beverage	During-exercise mouth-rinse
PL_PL	PL-Drink	PL
PL_CHO	PL-Drink	CHO
R_CHO	PL-Drink w/ CHO-Rinse	CHO

Preliminary Testing and Familiarization

To determine $\text{VO}_{2\text{max}}$, subjects performed an incremental exercise on a bicycle ergometer (Velotron Racermate, Inc., Seattle, Washington) while gas exchange was recorded on a Moxus Modular Metabolic System (AEI Technologies, Pittsburgh, Pennsylvania). After a five-minute warmup at a self-selected pace, the test began at 100-150 W and increased by 25 W every two-minutes until volitional exhaustion. Heart rate was recorded at the end of each stage using a Polar heart rate monitor and $\text{VO}_{2\text{max}}$ was recorded as the highest 30-second average value. Watt_{max} was documented as the workload of the last completed stage of the test. Body weight was recorded prior to the $\text{VO}_{2\text{max}}$ test, and used to calculate beverage volumes for the experimental protocol.

A familiarization trial was conducted to allow subjects to learn the experimental protocol and minimize variance between trials related to learning/training effects. The familiarization trial was identical to experimental trials (described below) with the exceptions that a) subjects did not report to the lab two hours prior to exercise, b) no pre-exercise beverage was provided, and c) no finger stick blood samples were obtained. During the familiarization trial, water was consumed *ad libitum* and a pedestal fan was set to the subject's preferred speed setting. Volume of water consumed and any changes in fan speed were recorded and held consistent throughout experimental trials. Individual seat and handlebar settings on the cycle ergometer were recorded and replicated throughout experimental trials.

Pre-Exercise Beverages and Mouth Rinse Solutions

Pre-exercise beverages (PL-drink) consisted of 10 ml/kg water, flavored with a non-caloric sweetener (all treatments). In the R_CHO trial, the PL-drink was followed by pre-exercise CHO-MR. 25 ml of CHO solution (15% maltodextrin, plus non-caloric sweetener) was rinsed in the mouth for five seconds and expectorated. Following a seven second pause, this procedure was repeated ten times, for a total of 250 ml over a period of two minutes. This

protocol was designed to mimic the mouth exposure to CHO during beverage consumption of the same volume.

MR utilized during exercise were 25 ml each. In PL_PL, the rinse consisted of water with a non-caloric sweetener. In the PL_CHO and R_CHO trials, the rinse was a 6.4% maltodextrin solution with the same non-caloric sweetener. MR were refrigerated until administered at minute 0 and 7.5 of constant-load cycling, and at 0-km, 5-km, 10-km, 15-km, 20-km, and 25-km of TT for a total of eight rinses, as shown in Figure 1. Subjects were instructed to vigorously rinse the 25-ml solution in mouth for five seconds and expectorate with no ingestion of solution at any point during the experimental protocol.

Experimental Protocol

Subjects arrived at the human performance laboratory after an overnight fast. After five minutes of rest, a finger stick blood sample (~0.25 ml) was obtained and subjects completed a satiety scale (appendix B). Immediately afterwards, the pre-exercise beverage was consumed. Blood was sampled again via finger sticks at 30 and 120 minutes after consumption of the pre-exercise beverage. Subjects completed 15 minutes of constant-load cycling at the following incremental workloads: 40% Watt_{max} (minute 0-4), 55% Watt_{max} (minutes 4-9), and 70% Watt_{max} (minutes 9-15). Subjects were allowed three minutes before starting the 30-km simulated time-trial. Subjects were instructed to give a maximal effort to complete 30-km in the shortest amount of time possible as if it was a competitive event. No feedback of performance was provided during experimental trials, except for distance completed.

Dietary and Exercise Control

Subjects recorded 24-hour dietary intake and 48-hour physical activity prior to each data collection period, before the first experimental time-trial (appendix C and D, respectively). Subjects were asked to refrain from strenuous exercise for 48 hours, alcohol and tobacco for 24

hours pre-trial and caffeine for 12 hours before experimental trial. An eight-ounce vanilla nutritional shake was provided as a snack before bedtime to ensure consistency in pre-exercise nutritional intake (appendix E). Scanned copies of dietary/exercise records were provided for subject to replicate for each subsequent experimental trial. Subjects were requested to maintain consistent diet, exercise, and sleep habits throughout the duration of the study.

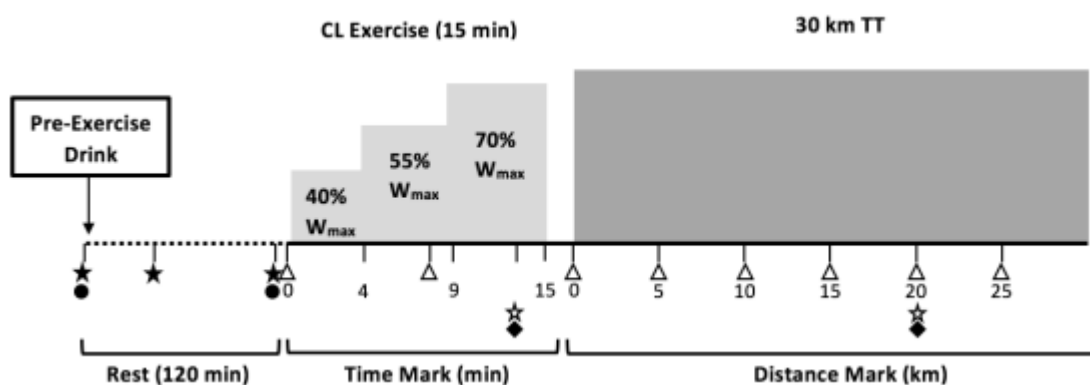


Figure 1. Diagram of experimental procedure. Symbols are as following:

- ★ Fingerstick Blood Draw (Glucose)
- Satiety Scale
- △ Mouth Rinse
- ◆ VO₂, V_E, RER, Fingerstick Blood Draw (Glucose and Lactate), HR, RPE, GI Distress

Performance

Time to complete the 30-km time-trial was recorded using the Coach Training Software (RacerMate Inc., Seattle, Washington).

Metabolic measurements

VO₂ (ml/min), ventilation (V_E) (L/min), and respiratory exchange ratio (RER) were recorded from minute ten to fifteen during constant-load cycling and for five minutes at 20-km of time-trial. The last three of five minutes of metabolic measurement were averaged to allow for

two minutes of breathing equilibration. All metabolic values were assessed using a MOXUS modular system.

Heart Rate

Heart rate was monitored throughout the trial, and recorded at minute 13 of constant-load exercise and 20-km of the time-trial.

Gastrointestinal Discomfort and Ratings of Perceived Exertion (RPE)

Subjects rated gastrointestinal discomfort using a 1-10 scale (appendix F). RPE was obtained using the Borg's 6-20 scale (appendix G), both measurements were taken at minute 13 of constant-load exercise and 20-km of the time-trial.

Blood Glucose and Lactate Concentrations

Blood glucose and lactate concentrations were assessed from finger stick blood samples for a total of five time points, [prior to pre-exercise beverage (min 0), 30 minutes following the pre-exercise beverage (min 30), and 120 minutes following the pre-exercise beverage (min 120), minute 13 of constant-load cycling, and at 20-km of the time-trial]. Each sample contained approximately 0.25 ml of blood, with glucose and lactate concentrations measured using the YSI 2300 STAT PLUS (YSI Inc., Yellow Springs, Ohio).

Satiety Scale

Subject rated hunger status and satiety on a visual analog scale (VAS) pre-treatment (min 0) and immediately prior to exercise (min 120). The 100mm VAS ranged from 0mm, "I have not hungry at all", to 100mm, "I have never been more hungry" (appendix B). Satiety was converted into a change score to be compared across treatments. The VAS assessment of appetite sensations in single test meal was adopted from Flint and colleagues (24).

Statistical Analyses

Magnitude-based inferences were used to compare treatment effects for each of the dependent variables, using methods described by Batterham and Hopkins (7). All data was log transformed to diminish the effects of non-uniformity. A threshold for the smallest worthwhile change was determined for each dependent variable. The smallest worthwhile change for 30-km time-trial performance was determined to be 0.3 x the coefficient of variation (CV) of sub-elite cyclists between repeated time-trials ($CV = 1.3\%$) which translated to a difference of 0.39% or a 13.2 second difference in performance in the current protocol. The smallest worthwhile change threshold for all other measurements was calculated as 0.2 x standard deviation (from the placebo trial). Using a published spreadsheet (64), mean treatment differences, 90% confidence intervals and percent likelihoods of treatments resulting in beneficial/trivial/harmful effects in the population were calculated (7, 33). In addition, semantic inferences regarding the likelihood of observed effects resulting in beneficial/trivial/harmful effects in the population were determined, using the following guidelines: $< 1\%$ = almost certainly no chance, $1-5\%$ = very unlikely, $5-25\%$ = unlikely, $25-75\%$ = possible, $75-95\%$ = likely, $95-99\%$ = very likely, and $> 99\%$ = almost certain. Mechanistic/qualitative inferences were used to classify the effects of the treatment on all dependent variables, other than performance time. If the 90% confidence interval surpassed minimum thresholds for benefit and harm, the effect was classified as “unclear”. For performance times, clinical inferences were provided based on threshold chances of harm and benefit of 0.5% and 25%, respectively (and declaring beneficial when odds ratio of benefit/harm was > 66).

Chapter III

Manuscript

Mouth Exposure to Carbohydrate Prior to Exercise Possibly Impairs the Efficacy of Carbohydrate

Mouth Rinsing during Exercise

Abstract-

Decker K. P., M. J. Saunders, N. D. Luden, C. J. Womack, and N. J. Hladick. Mouth Exposure to Carbohydrate Prior to Exercise Possibly Impairs the Efficacy of Carbohydrate Mouth Rinsing during Exercise **Purpose:** Carbohydrate mouth-rinsing (CHO-MR) during intense endurance exercise has been associated with improved cycling performance, due to neurological influences. However, prior studies have reported the efficacy of CHO-MR is attenuated following a pre-exercise meal. To determine if this outcome is related to desensitization of CHO receptors (rather than metabolic effects following digestion), this study will investigate whether CHO-MR prior to exercise influences cycling performance when CHO-MR is also used during exercise. **Methods:** Eight trained cyclists (age, 24 ± 6 yr; height, 176 ± 6 cm; weight 75 ± 12 kg; VO_{2max} , 61 ± 8 ml/kg/min) completed three exercise trials, each consisting of 15-min of incremental, constant-load exercise followed by a simulated 30-km time-trial (TT). Treatment beverages in the trials were randomly counterbalanced: a) PL_PL: placebo before and during exercise, b) PL_CHO: placebo pre-exercise, CHO-MR during exercise, and c) R_CHO: CHO-MR before and during exercise. Physiological responses (VO_2 , V_E , RER, RPE, heartrate, blood glucose and lactate) were assessed during constant-load exercise and during the TT. Magnitude-based qualitative inferences were used to evaluate differences in responses between treatments. **Results:** TT performance was 'possibly' impaired (59% likelihood) with R_CHO (57.3 ± 3.6) versus PL_CHO (56.9 ± 3.0 min). Both trials were 'likely' slower than PL_PL (55.8 ± 3.1 min), but the reliability of performance data from this trial may have been impacted by measurement error, which limited our ability to determine the influence of CHO-MR during exercise. Physiological responses between treatments during constant-load cycling, and the TT were generally similar between all treatments. **Conclusion:** A pre-exercise CHO-MR had a possibly negative impact on cycling performance that also included CHO-MR during exercise. Although further evidence is required to validate this finding, our data suggests that desensitization of CHO receptors related to recent CHO exposure may be partially responsible for previous reports that the efficacy of CHO-MR during exercise are attenuated by pre-exercise feedings. **Keywords:** CYCLING, CARBOHYDRATE, MOUTH-RINSING, PERFORMANCE, ERGOGENIC-AIDS.

Introduction

Carbohydrate (CHO) ingestion during prolonged exercise has been widely reported to enhance performance. Since endogenous CHO stores can be limiting during exercise durations > 90 minutes, the ergogenic effects of CHO ingestion during prolonged exercise are predominantly attributed to maintenance of blood glucose (33), muscle and liver glycogen sparing (18, 19, 30, 31) and higher CHO oxidation late in exercise (18, 19, 39, 65). More recently, CHO ingestion has also been found to be ergogenic during shorter, high intensity aerobic exercise (~ 60 min, >75% $\text{VO}_{2\text{max}}$) (2, 11, 22, 38, 46), though not all studies agree (20, 44, 48). However, because CHO availability is not considered to be limiting under these conditions (38, 44, 55), and minimal exogenous CHO are oxidized at such intense exercise intensities (15, 38, 40, 44), it is believed that other mechanisms are responsible for the ergogenic effects of CHO during intense aerobic exercise of approximately one hour.

Carter and colleagues reported that CHO mouth-rinsing (MR) without ingestion enhanced time-trial (TT) performance lasting approximately one hour by ~ 3% (14). Other studies have confirmed the ergogenic effect of CHO-MR on TT performance of approximately one-hour (7, 16, 21, 23, 27, 41, 50, 52), yet not all studies report enhanced performance (1, 10, 36, 60, 63). It was determined that CHO is detected by oral-pharyngeal receptors in the mouth to send feedback to the central nervous system (CNS) (4), stimulating the motor cortex and dopaminergic pathways (3, 26, 28, 57). Therefore the ergogenic effects of CHO ingestion (and MR) during intense aerobic exercise are related to increased motor output, improved motivational drive, and reduced neuromuscular fatigue or perceived exertions, (7, 13, 16, 28, 37).

The efficacy of CHO-MR may be influenced by various factors, including: MR time, with most studies using a five second MR (10, 14, 16, 27, 49, 50, 52–54); CHO concentration, with most studies using a 6.4% CHO solution (10, 14, 17, 23, 47, 50, 52, 58); and total number of MR, with most studies using ≥ 8 during exercise protocols of approximately one hour (14, 16, 23, 41, 50, 63). Perhaps more important is the influence that pre-exercise nutritional status has on the

efficacy of CHO-MR during exercise. A majority of studies have found CHO-MR enhances performance when exercise is conducted in a fasted state (14, 16, 41, 52, 54), and some studies report that CHO-MR efficacy is attenuated in a CHO fed-state (10, 23, 41). Lane and colleagues reported CHO-MR improved a simulated 60-minute cycling trial by 3.4% after an overnight fast, but only 1.8% in a CHO fed state (41). Similarly, Fares and colleagues observed CHO-MR improved time to exhaustion performance by 5.6 minutes when fasted, but only 1.9 minutes when fed a CHO rich breakfast (23). The pre-exercise CHO feedings may modulate the CNS response to CHO-MR as more neural activations has been shown in a physiological state of hunger (32, 51), presumably due to homeostatic signals related to satiety (56).

The mechanism for the attenuation in exercise performance with CHO-MR following pre-exercise CHO feedings is not fully understood. It is unclear whether this outcome is related to altered metabolic responses following digestion (i.e. such as changes in blood glucose, insulin, ghrelin, etc.), or due to neural influences related to desensitization of (or down-regulation of feedback from) oral-pharyngeal CHO receptors. To provide information regarding the neural effects of pre-exercise CHO, the purpose of this study was to determine the influence of a pre-exercise CHO-MR on the ergogenic effects of CHO-MR during cycling. It was hypothesized the pre-exercise CHO-MR would attenuate the neurological influences of CHO-MR during exercise, resulting in impaired cycling performance compared to no pre-exercise CHO exposure.

Methods

Subjects

Eight trained cyclists were recruited from James Madison University and the surrounding area in Harrisonburg, Virginia. Subjects met the following inclusion criteria: 18 - 45 years of age, ≥ 2 years of experience in endurance cycling events, $VO_{2max} \geq 50$ ml/kg/min, consistent training prior to this study of ≥ 3 days/week, and ≥ 4 training sessions ≥ 2 hours in duration over the past 2 months. Subjects were classified as low-risk for health complications according to

ACSM guidelines (59), and provided written informed consent prior to starting the study.

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R_CHO	PL-Drink w/ CHO-Rinse	CHO

Preliminary Testing and Familiarization

To determine $\text{VO}_{2\text{max}}$, subjects performed an incremental exercise on a bicycle ergometer (Velotron Racermate, Inc., Seattle, Washington) while gas exchange was recorded on a Moxus Modular Metabolic System (AEI Technologies, Pittsburgh, Pennsylvania). After a five-minute warmup at a self-selected pace, the test began at 100-150 W and increased by 25 W every two-minutes until volitional exhaustion. Heart rate was recorded at the end of each stage using a Polar heart rate monitor and $\text{VO}_{2\text{max}}$ was recorded as the highest 30-second average value. Watt_{max} was documented as the workload of the last completed stage of the test. Body weight was recorded prior to the $\text{VO}_{2\text{max}}$ test, and used to calculate beverage volumes for the experimental protocol.

A familiarization trial was conducted to allow subjects to learn the experimental protocol and minimize variance between trials related to learning/training effects. The familiarization trial was identical to experimental trials (described below) with the exceptions that a) subjects did not report to the lab two hours prior to exercise, b) no pre-exercise beverage was provided, and c) no finger stick blood samples were obtained. During the familiarization trial, water was consumed *ad libitum* and a pedestal fan was set to the subject's preferred speed setting. Volume of water consumed and any changes in fan speed were recorded and held consistent throughout experimental trials. Individual seat and handlebar settings on the cycle ergometer were recorded and replicated throughout experimental trials.

Pre-Exercise Beverages and Mouth Rinse Solutions

Pre-exercise beverages (PL-drink) consisted of 10 ml/kg water, flavored with a non-caloric sweetener (all treatments). In the R_CHO trial, the PL-drink was followed by pre-exercise CHO-MR. 25 ml of CHO solution (15% maltodextrin, plus non-caloric sweetener) was rinsed in the mouth for five seconds and expectorated. Following a seven second pause, this procedure was repeated ten times, for a total of 250 ml over a period of two minutes. This protocol was designed to mimic the mouth exposure to CHO during beverage consumption of the same volume.

MR utilized during exercise were 25 ml each. In PL_PL, the rinse consisted of water with a non-caloric sweetener. In the PL_CHO and R_CHO trials, the rinse was a 6.4% maltodextrin solution with the same non-caloric sweetener. MR were refrigerated until administered at minute 0 and 7.5 of constant-load cycling, and at 0-km, 5-km, 10-km, 15-km, 20-km, and 25-km of TT for a total of eight rinses, as shown in Figure 1. Subjects were instructed to vigorously rinse the 25-ml solution in mouth for five seconds and expectorate with no ingestion of solution at any point during the experimental protocol.

Experimental Protocol

Subjects arrived at the human performance laboratory after an overnight fast. After five minutes of rest, a finger stick blood sample (~0.25 ml) was obtained and subjects completed a satiety scale. Immediately afterwards, the pre-exercise beverage was consumed. Blood was sampled again via finger sticks at 30 and 120 minutes after consumption of the pre-exercise beverage. Subjects completed 15 minutes of constant-load cycling at the following incremental workloads: 40% Watt_{max} (minute 0-4), 55% Watt_{max} (minutes 4-9), and 70% Watt_{max} (minutes 9-15). Subjects were allowed three minutes before starting the 30-km simulated TT. Subjects were instructed to give a maximal effort to complete 30-km in the shortest amount of time possible as if it was a competitive event. No feedback of performance was provided during experimental trials, except for distance completed.

Dietary and Exercise Control

Subjects recorded 24-hour dietary intake and 48-hour physical activity prior to each data collection period, before the first experimental TT. Subjects were asked to refrain from strenuous exercise for 48 hours, alcohol and tobacco for 24 hours and caffeine for 12 hours before experimental trial. A nutritional shake was provided as a snack before bedtime to ensure consistency in pre-exercise nutritional intake. Scanned copies of dietary/physical activity records were provided for subject to replicate for each subsequent experimental trial. Subjects were requested to maintain consistent diet, exercise, and sleep habits throughout the duration of the study.

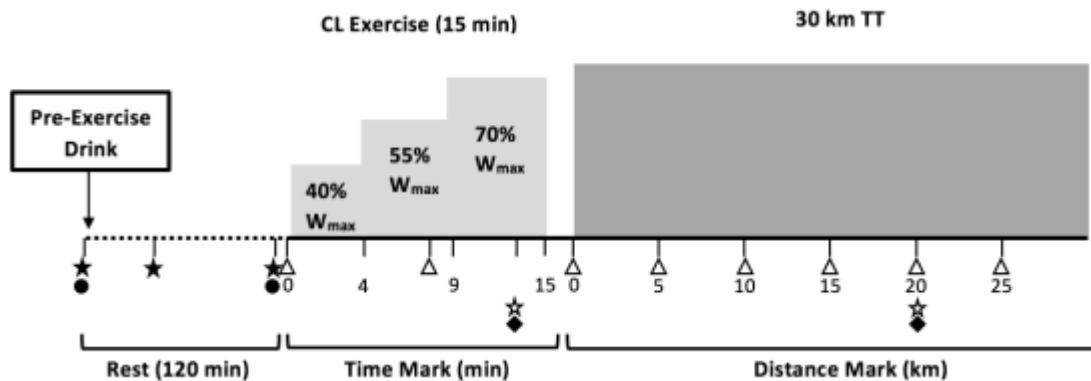


Figure 1. Diagram of experimental procedure. Symbols are as following:

- ★ Fingerstick Blood Draw (Glucose)
- Satiety Scale
- △ Mouth Rinse
- ◆ VO_2 , V_E , RER, Fingerstick Blood Draw (Glucose and Lactate), HR, RPE, GI Distress

Performance

Time to complete the 30-km TT was recorded using the Coach Training Software (RacerMate Inc., Seattle, Washington).

Metabolic measurements

VO_2 (ml/min), ventilation (V_E) (L/min), and respiratory exchange ratio (RER) were recorded from minute ten to fifteen during constant-load cycling and for five minutes at 20-km of TT. The last three of five minutes of metabolic measurement, starting at minute 13, were averaged, allowing two minutes for breathing equilibration. All metabolic values were assessed using a Moxus Modular Metabolic System.

Heart Rate

Heart rate was monitored throughout the trial, and recorded at minute 13 of constant-load exercise and at 20-km of the TT.

Gastrointestinal Discomfort and Ratings of Perceived Exertion (RPE)

Subjects rated gastrointestinal discomfort using a 1-10 scale. RPE was obtained using the Borg's 6-20 scale at minute 13 during constant-load exercise and 20-km of the TT.

Blood Glucose and Lactate Concentrations

Blood glucose and lactate concentrations were assessed from finger stick blood samples for a total of five time points, [prior to pre-exercise beverage (min 0), 30 minutes following the pre-exercise beverage (min 30), and 120 minutes following the pre-exercise beverage (min 120), at minute 13 of constant-load, and at 20-km of TT]. Each sample contained approximately 0.25 ml of blood, with glucose and lactate concentrations measured using the YSI 2300 STAT PLUS (YSI Inc., Yellow Springs, Ohio).

Satiety Scale

Subject rated hunger status and satiety on a visual analog scale (VAS) pre-treatment (min 0) and immediately prior to exercise (min 120). The 100mm VAS ranged from 0mm, "I have not hungry at all", to 100mm, "I have never been more hungry". Satiety was converted into a change score to be compared across treatments. The VAS assessment of appetite sensations in single test meal was adopted from Flint and colleagues (25).

Statistical Analyses

Magnitude-based inferences were used to compare treatment effects for each of the dependent variables, using methods described by Batterham and Hopkins (8). All data was log transformed to diminish the effects of non-uniformity. A threshold for the smallest worthwhile change was determined for each dependent variable. The smallest worthwhile change for 30-km TT performance was determined to be 0.3 x the coefficient of variation (CV) of sub-elite cyclists

between repeated TT ($CV = 1.3\%$) which translated to a difference of 0.39% or 13.2 second difference in performance in the current protocol. The smallest worthwhile change threshold for all other measurements was calculated as $0.2 \times$ standard deviation (from the placebo trial). Using a published spreadsheet (64), mean treatment differences, 90% confidence intervals and percent likelihoods of treatments resulting in beneficial/trivial/harmful effects in the population were calculated (8, 35). In addition, semantic inferences regarding the likelihood of observed effects resulting in beneficial/trivial/harmful effects in the population were determined, using the following guidelines: $< 1\%$ = almost certainly no chance, $1-5\%$ = very unlikely, $5-25\%$ = unlikely, $25-75\%$ = possible, $75-95\%$ = likely, $95-99\%$ = very likely, and $> 99\%$ = almost certain. Mechanistic/qualitative inferences were used to classify the effects of the treatment on all dependent variables, other than performance time. If the 90% confidence interval surpassed minimum thresholds for benefit and harm, the effect was classified as “unclear”. For performance times, clinical inferences were provided, based on threshold chances of harm and benefit of 0.5% and 25%, respectively (and declaring beneficial when odds ratio of benefit/harm was > 66).

Results

Eleven endurance-trained cyclists from James Madison University and the Harrisonburg, VA area volunteered to participate in this study. Two subjects withdrew before completion because of circumstances unrelated to the study and one subject was dropped due to non-compliance to maximal effort during TT. This resulted in six male and two female cyclists (age, 24 ± 6 years; height, 176 ± 6 cm; weight 75 ± 12 kg; BMI, 24.3 ± 2.6 ; VO_{2max} , 61 ± 8 ml/kg/min; $Watt_{max}$ 316 ± 58 W). All experimental procedures were under similar environmental conditions (21.8 ± 1.1 °C, $24.4 \pm 11.3\%$ humidity, 728.6 ± 4.9 mmHg).

Pre-exercise Measurements

Upon arrival to the lab, fasted blood glucose was similar between PL_PL (84.0 ± 8.3 mg/dL), PL_CHO (83.0 ± 6.4 mg/dL), and R_CHO (79.7 ± 5.7 mg/dL), with R_CHO values ‘possibly’ lower versus PL_CHO. Changes in blood glucose over the 120 minutes prior to exercise were negligible, with no clear differences between PL_PL (-1.3 ± 6.3 mg/dL), PL_CHO (-4.0 ± 7.2 mg/dL) and R_CHO (-0.4 ± 4.6 mg/dL), respectively. Hunger rating change scores increased from pre-treatment (minute 0) to pre-exercise (minute 120) to a similar degree between for PL_PL (20 ± 12.8), PL_CHO (15.6 ± 9.1), and R_CHO (18.3 ± 16.7).

Responses during Constant-load Exercise

Metabolic measurements obtained during constant-load cycling at 70% W_{\max} are displayed in Table 2. VO_2 responses during PL_PL and PL_CHO were ‘possibly’ higher than R_CHO. Blood glucose responses during PL_PL and R_CHO were ‘likely’ lower than PL_CHO. Treatment effects for all other physiological responses were ‘unclear’.

Table 2. Physiological Responses during Cycling at 70% W_{\max} (Mean \pm SD)

Variable	PL_PL	PL_CHO	R_CHO
Heart rate (bpm)	158 ± 13	151 ± 12	149 ± 11
VO_2 (ml/min)	$3349 \pm 510^*$	$3306 \pm 558^*$	3219 ± 608
Ventilation (L/min)	91.5 ± 15.9	92.0 ± 15.7	92.1 ± 17.0
RER	0.93 ± 0.06	0.93 ± 0.08	0.93 ± 0.06
RPE (6-20)	12.8 ± 1.3	12.3 ± 2.0	13.6 ± 1.0
Glucose (mg/dL)	$73.3 \pm 13.6^{\$}$	79.8 ± 9.5	$75.3 \pm 7.4^{\$}$
Lactate (mmol/L)	2.52 ± 1.32	2.33 ± 1.35	2.55 ± 0.63

PL_PL = Placebo before and during exercise; PL_CHO = Placebo pre-exercise, carbohydrate mouth-rinse during exercise; R_CHO = carbohydrate mouth-rinse before and during exercise
 $*$ = ‘Possibly’ higher than R_CHO; $\$$ = ‘Likely’ lower than PL_CHO

Responses during Cycling TT

VO₂ responses during R_CHO were ‘likely’ lower than PL_PL. RPE responses during R_CHO were ‘likely’ higher than PL_PL. Treatment effects for all other variables were ‘unclear’.

Table 3. Physiological Responses during Cycling Time-Trial (Mean \pm SD)

Variable	PL_PL	PL_CHO	R_CHO
Heart rate (bpm)	161 \pm 13	162 \pm 14	164 \pm 13
VO ₂ (ml/min)	3085 \pm 358	2986 \pm 436	2895 \pm 505 [#]
Ventilation (L/min)	81.5 \pm 11.3	78.7 \pm 9.1	75.4 \pm 9.5
RER	0.83 \pm 0.03	0.83 \pm 0.04	0.83 \pm 0.03
RPE (6-20)	15.5 \pm 1.1	15.6 \pm 1.6	16.0 \pm 0.9 ^{&}
Glucose (mg/dL)	74.0 \pm 18.1	74.9 \pm 20.0	75.4 \pm 6.5
Lactate (mmol/L)	2.27 \pm 1.13	2.30 \pm 0.65	2.03 \pm 1.09

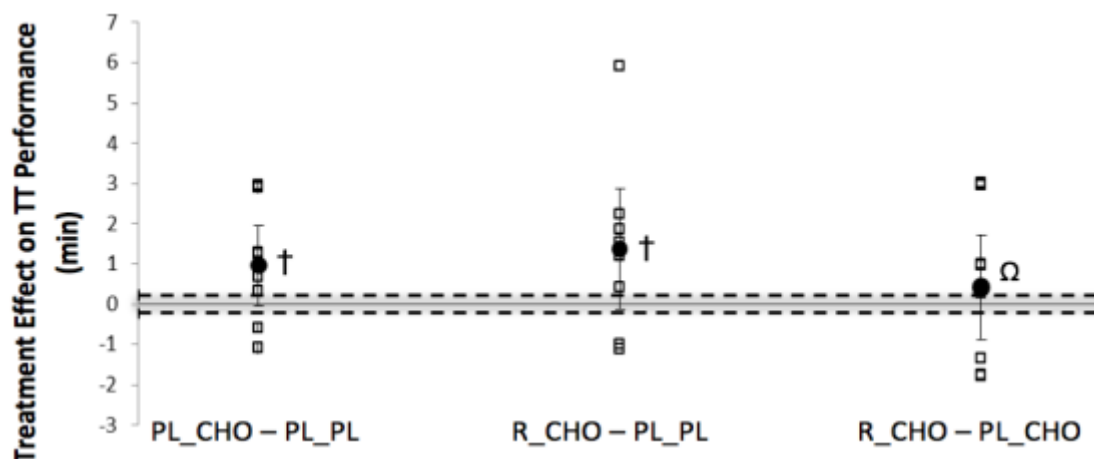
PL_PL = Placebo before and during exercise; PL_CHO = Placebo pre-exercise, carbohydrate mouth-rinse during exercise; R_CHO = carbohydrate mouth-rinse before and during exercise
[#] = ‘Likely’ lower than PL_PL; [&] = ‘Likely’ higher than PL_PL

Treatment Effects on Performance

Cycling performance times during the 30-km TT were as follows: PL_PL: 55.8 \pm 3.1 min; PL_CHO: 56.9 \pm 3.0 min; R_CHO: 57.3 \pm 3.6 min. Mean differences (and individual responses) in performance between treatments (\pm 90% CI) are illustrated in Figure 2.

Performance in the PL_PL trial was ‘likely’ beneficial compared to R_CHO (% likelihoods of beneficial/trivial/harmful effect: 91/5/4) and PL_CHO (% likelihoods: 91/7/3). Performance in R_CHO was ‘possibly’ harmful than PL_CHO (% likelihoods: 20/21/59). Treatment effects were also examined after statistically adjusting for treatment order, but this did not alter any of the reported inferences.

Figure 2. Treatment Effects on 30-km TT Performance (Mean \pm 90%CI)



PL_PL = Placebo before and during exercise; PL_CHO = Placebo pre-exercise, carbohydrate mouth-rinse during exercise; R_CHO = carbohydrate mouth-rinse before and during exercise. Filled circles represent the mean value, open squares represent individual scores (some are obscured by the mean value), Dashed lines represent the threshold value for a meaningful effect (\pm 13.2 seconds). † = 'Likely' faster for PL_PL; Ω = 'Possibly' harmful for R_CHO

GI distress symptoms

The symptoms of GI distress remained low in all trials (average values were < 2.0 for all individual symptoms, at all-time points). Only one subject reported upper-GI distress symptoms ≥ 5 ("severe" or higher) during exercise. This subject experienced 'stomach problems' during constant-load exercise and during the TT of the R_CHO treatment.

Discussion

The aim of this study was to determine if pre-exercise CHO exposure in the mouth influenced the efficacy of CHO-MR during a 30-km cycling performance TT. Our hypothesis was that pre-exercise CHO-MR would attenuate the neurological influences of CHO-MR during exercise, resulting in impaired performance compared to no pre-exercise CHO exposure. The primary finding was that CHO-MR two hours before exercise (R_CHO) had a 'possibly' harmful effect on performance compared to no CHO-MR before exercise (PL_CHO). Although the outcome requires some verification in future studies, this is the first evidence suggesting that

CHO sensed in the mouth (without ingestion) prior to exercise might attenuate the efficacy of CHO-MR during cycling.

The performance outcome in the PL_PL trial (i.e. ‘likely’ faster 30-km TT than the trials in which CHO-MR was performed) was surprising, and may have been influenced by measurement error and/or researcher error. Body weight was incorrectly entered in the ergometer software prior to one PL_PL TT, and this error (and a similar issue in another trial), may have contributed to the relatively fast time reported in the PL_PL trial. Performance outcomes were reassessed after removal of these subjects, with minimal impact on the treatment effects reported in our results. This suggests that the CHO-MR during exercise did not have a positive impact on performance in our study. However, the decrease in sample size and statistical power resulting from removal of these subjects minimized the confidence with which we could infer meaningful conclusions regarding this data. As such, further discussion of our results will focus mainly on the comparison of the pre-exercise CHO-MR on 30-km TT between the PL_CHO and R_CHO trials (from the full complement of eight subjects).

It is known that rinsing the mouth with CHO generates gustatory sensory information for CNS integration (4). Specifically, fMRI and transcranial magnetic stimulation studies have shown the insula/frontal operculum, orbitofrontal cortex, ventral striatum, and anterior cingulate cortex regions of the brain relating to the pleasure/reward and motor controls are stimulated during the presence of CHO in the mouth (16, 26, 28, 32). Our finding that R_CHO ‘possibly’ attenuated the efficacy of CHO-MR during exercise is provocative (despite its moderate statistical certainty) because it suggests that oral-pharyngeal CHO receptors may have been desensitized by prior exposure to CHO. If this occurred, a reduction in motivation, motor output, and ultimately TT performance would be expected from the CHO-MR during exercise.

An alternative explanation for the ‘possibly’ impaired 30-km TT performance in R_CHO could be due to desensitization of dopamine pathways. The presence of CHO in the mouth induces dopaminergic pathways in the brain rewards center (3), playing a role in motivational

behavioral (12) and maintaining mechanical efficiency during exercise (29). The CHO-MR prior to exercise was expected to stimulate the release of dopamine, which may have altered the dopamine response related to the subsequent CHO-MR during cycling. There is evidence that repeated stimulation from food/carbohydrate can spike dopamine levels (6) and lead to alterations in the dopamine reward circuitry for secondary exposure of the same stimuli (62). As such, , the potentially slower TT performance in R_CHO could be related to a blunted dopamine response to the subsequent CHO-MR, compromising mechanical efficiency and/or motivation during cycling (29). However, this theory is highly speculative, and further study is required to determine the influence of CHO-MR on the dopaminergic pathways.

The neurophysiological explanation for a ‘possible’ treatment differences in performance is supported by the absence of CHO ingestion in all trials. The pre-exercise CHO-MR did not impact blood glucose levels in the 120-min recovery period prior to exercise. However, there was a ‘likely’ elevated blood glucose response in PL_CHO versus R_CHO and PL_CHO during constant-load cycling. Although the volume of CHO-MR expectorated was not measured, researchers were present to make sure carbohydrate was not ingested. In addition, our CHO-MR consisted of maltodextrin which cannot be digested in the mouth (34). Other studies have reported elevated blood glucose responses with CHO-MR during cycling (5, 42), and suggested that this was caused by sympathetic neural activity inducing hepatic glucose release (45). The lack of increase in blood glucose from a CHO-MR in R_CHO could potentially be explained by a desensitization of oral-pharyngeal receptors from the pre-exercise CHO-MR.

There is some evidence suggesting the activation of reward centers in the brain from CHO in the mouth is related to the degree of hunger (16, 32). All trials in the present study were conducted after an overnight fast, and hunger ratings upon arrival to the lab, and prior to exercise were similar between treatments. Based on this evidence, varying degrees of hunger does not appear to explain the possible differences in performance observed between our treatments. However, it is possible that varying levels of hunger could partially explain the attenuated

ergogenic effects reported in prior studies when using CHO-MR during exercise in the fed versus fasted state (10, 23, 41). Following ingestion of CHO, homeostatic signals such as glucose, insulin, ghrelin, and leptin are altered which may modulate satiety and sensory input of CHO-MR during exercise to the CNS (9, 24, 43). Further study is required to determine the influence of hunger status and homeostatic signals on the effects of CHO-MR during exercise.

Among the eight subjects to complete the study, five recorded their worst 30-km TT performance with the R_CHO treatment. R_CHO reduced the mean TT performance by 24 seconds or 0.7% compared to PL_CHO, which exceeded the “smallest worthwhile change” threshold of 13.2 seconds or 0.4% (35). Although the apparent effect of the pre-exercise CHO-MR on subsequent performance was relatively small, it is possible that the time-period between the CHO-MR and onset of exercise could have provided sufficient time for receptor sensitivity in the mouth to return to near baseline (fasted) levels. Future research investigating the time course of neural responses to CHO exposure in the mouth would be of interest to further understand the influences of CHO feedings and CHO-MR. For example, future studies could investigate the effects of frequent CHO-MR leading up to exercise, to maximize the potential of any neural desensitizing effect that may occur, prior to TT performance using a CHO-MR.

It is worth acknowledging the current study was part of a larger project, which also investigated the effects of pre-exercise feedings with different glycemic indices. Thus, subjects completed the experimental protocols on five occasions (plus an additional familiarization trial), lasting 5-6 weeks. An analysis of the effects of trial-order revealed no systematic influences on the treatment outcomes in this study, but it is possible that the prolonged nature of the study could have increased the between-trial variability in TT performance (due to changes in motivation, fitness, etc. over the course of the study). This could have reduced the sensitivity of our exercise tests to detect small but important changes in performance between treatments. In addition, the previously noted concerns regarding the reliability of the PL_PL performance trials prevented us from determining the ergogenic effect of CHO-MR in the fasted state (i.e. % difference between

PL_PL and PL_CHO). Therefore, we cannot reliably infer the degree to which R_CHO may have attenuated the ergogenic effects of CHO-MR during exercise.

In summary, this is the first study to provide some evidence that mouth exposure to carbohydrate (without ingestion) prior to exercise might attenuate the efficacy of CHO-MR during cycling. We observed that pre-exercise CHO mouth exposure ‘possibly’ harms TT performance when CHO-MR are used during exercise. Further study is required to validate this finding, due to its moderate statistical certainty (59% likelihood of harm). If verified, this outcome suggests that the attenuated ergogenic effects of CHO-MR during exercise in the fed state may be the result of desensitization of oral-pharyngeal receptors (and/or neural output) due to prior CHO exposure.

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Appendix A
Informed Consent

James Madison University – Department of Kinesiology

Informed Consent

Purpose

You are being asked to volunteer for a research project conducted by Nikolai Hladick, Kevin Decker, Dr. Nick Luden, Dr. Mike Saunders, and Dr. Christopher Womack from James Madison University titled *Impact of pre-exercise carbohydrate exposure, and the glycemic index of pre-exercise feeding on the ergogenic effects of carbohydrate mouth-rinsing during cycling*

The primary goals of this study are to determine the effects of a) pre-exercise exposure to carbohydrate, and b) pre-exercise beverages of differing glycemic indexes on high intensity cycling performance when a carbohydrate mouth rinse is used during exercise.

Experimental Procedures

You will be asked to report to James Madison University's Human Performance Laboratory (Godwin 209) on 7 occasions, each separated by at least 7 days. These include one initial testing session, one familiarization trial, and five experimental exercise trials. The initial testing session will last approximately 1 hour and the familiarization trial will last approximately 1 hour and 30 minutes. Each experimental exercise trial will require approximately 3.5 hours. The total time commitment will be approximately 20 hours.

Initial Exercise Testing Session – Visit 1 – 1 hour

You will be asked to complete short questionnaires related to your health history and exercise training, to determine whether you meet the criteria for participation and to rule out any health-related risk factors that would prevent you from participating in this study. During this process, you will be asked to share information concerning your lifestyle, training habits, and general health with the researchers. If you meet the participation criteria, your height and body weight will be measured and your maximal oxygen consumption (VO_{2max}) will be assessed with a test on a cycle ergometer. You will begin this test by cycling at a moderate intensity, after which the workload will be increased by 25 watts every 2 minutes until you are unable to continue due to fatigue (~10-20 min). Throughout the trial, you will breathe through a mouthpiece that is connected to a metabolic cart, in order to measure your oxygen consumption and other variables during exercise. Heart rate will be also be monitored continuously by a wearable heart rate monitor on your chest.

Familiarization Trial – Visit 2 – 1 hour and 30 minutes

During the familiarization trial you will be asked to complete a simulated 30 km cycling time trial on a cycle ergometer (~ 50 min). During the time trial you will be asked to rinse your mouth with water for 5 seconds every 5 km without swallowing. On one occasion during the trial (20 km), you will have your oxygen consumption measured for 5 minutes, by wearing the mouthpiece described above. You will also be asked to rate your perceived effort and gastrointestinal discomfort (using a scale provided by the researchers) at these time-points. Heart rate will be measured continuously via a wearable heart rate monitor on your chest.

Experimental Trials – Visits 3 through 7 – 3.5 hours each

You will report to the laboratory after an overnight fast (no food after dinner the night prior to the trial), and provide a small (0.25 ml) blood sample from a finger-prick. Following the blood sample, you will consume a sports beverage, and then rest for two hours, during which time two

additional 0.25 ml blood samples will be obtained. After the rest period, 15 minutes of cycling at moderate intensity will be completed, followed immediately by a simulated 30 km cycling time trial, as described above. You will be asked to give a maximal effort during each time trial and to treat it as a competitive event. During each trial, you will also be asked to rinse your mouth with a sports drink for 5 seconds every 5 km without swallowing. You will receive all the measurements described in the Familiarization Trial above (oxygen consumption, heart rate, perceived effort and gastrointestinal discomfort). You will also receive finger-pricks at two time-points to obtain small blood samples (0.125 ml) from your finger. Each of the five experimental trials will include a different combination of pre-exercise sports drink (or mouth rinse) and/or sports drink mouth rinse during exercise. The order in which you receive the different beverages and mouth rinses during the experimental trials will be randomly assigned.

Dietary and Exercise Controls

You will be asked to record your food intake for 24 hours prior to each experimental visit. After bringing the initial dietary record to the Human Performance Laboratory, you will be given a copy, and will be asked to replicate your food intake for the 24 hours before each subsequent visit. You will also be asked to record your physical activity/exercise during the 72 hours prior to each experimental trial and to maintain consistent physical activity/exercise patterns between trials. You will be asked to refrain from heavy exercise 48 hours pre-trial, alcohol and tobacco 24 hours pre-trial, caffeine 12 hours pre-trial, and will be asked to fast the night before each experimental visit (no food after dinner).

Risks

The risks associated with maximal exercise and maximal exercise testing are minimal in individuals who are considered healthy and at low risk for cardiovascular disease and cardiac events according to the American College of Sports Medicine. In order to participate in this study, you must be considered low risk after initial assessment via health history questionnaires. You are expected to be honest when filling out questionnaires and identifying any risk factors you may have. In the case of a cardiac or emergency event during exercise, an emergency plan is in place, including access to a phone to contact emergency personnel. At least one investigator at each testing session will be CPR certified, and an AED is present in the laboratory.

The cycling time trials may induce muscle fatigue and soreness both immediately after the trial and for 1-2 days following the visit. Gastrointestinal distress is a possibility when consuming sports drinks before intense exercise. However, this poses no threat to your health or safety, and will at most cause mild discomfort. In addition, you may stop exercising at any point throughout the trials. The risks of blood sampling include slight discomfort, temporary minor bleeding, possibility for infection, and the possible transfer of blood-borne pathogens. Risks during blood sampling are considered to be minimal and OSHA safety protocols will be followed when handling blood samples. The researchers have completed JMU blood-borne pathogen training. In addition, the total amount of blood obtained throughout the study is very small [~ 2 ml per trial = 10 ml or < 0.4 fluid ounces, which is 2% of the amount given when donating blood in a single session (approximately 1 pint, or 473 ml)].

Benefits

Participating in this study includes receiving a free assessment of maximal oxygen consumption (which typically cost $> \$100$ at commercial testing facilities). You will also be contributing to the first study investigating the effects of pre-exercise mouth rinsing and pre-exercise meal GI on the efficacy of carbohydrate mouth rinsing during cycling. In addition, participants will receive a monetary incentive of \$200 for completion of the study. Participants who do not complete the

entire study will receive a prorated payment of \$35 for each of the experimental time-trials completed (i.e. trials 3-7 above).

Inquiries

If you have any questions or concerns, please contact Dr. Mike Saunders at saundemj@jmu.edu and (540) 568-8121 or Dr. Nicholas Luden at ludennd@jmu.edu and (540) 568-4069.

Questions about Your Rights as a Research Subject

Dr. David Cockley
Chair, Institutional Review Board
James Madison University
(540) 568-2834
cocklede@jmu.edu

Confidentiality

Data obtained in this study will be kept confidential and your name will not be identified with individual data. An identification code will be assigned to each participant in order to avoid identifying participant names with data, which will be kept in a locked cabinet. Once the study has been completed, any information connecting participants to their information/data will be destroyed. The researchers retain the right to use and publish non-identifiable data. Final aggregate results will be made available to you upon request.

Freedom of Consent

Your participation is entirely voluntary. You are free to choose not to participate. Should you choose to participate, you can withdraw at any time without consequences of any kind.

I have read this consent form and I understand what is being requested of me as a participant in this study. I freely consent to participate. I have been given satisfactory answers to my questions. The investigator provided me with a copy of this form. I certify that I am at least 18 years of age.

_____ Name of Subject (Printed)	_____ Name of Researcher (Printed)
_____ Name of Subject (Signed)	_____ Name of Researcher (Signed)
_____ Date	_____ Date

Appendix B

Satiety Scale

Subject Number _____

Trial Number _____

Date _____

I am not hungry at all	How hungry do you feel?	I have never been more hungry
I am completely empty	How satisfied do you feel?	I cannot eat another bite
Not at all full	How full do you feel?	Totally full
Nothing at all	How much do you think you can eat?	A lot
Yes, very much	Would you like to eat something sweet?	No, not at all
Yes, very much	Would you like to eat something salty?	No, not at all
Yes, very much	Would you like to eat something savoury?	No, not at all
Yes, very much	Would you like to eat something fatty?	No, not at all

0mm

100mm

Mark with a "O" at Minute ZERO

Mark with a "X" at Minute 120

Appendix C
24-Hour Diet Record

24-HOUR DIET RECORD

Subject number _____ Date _____ Day of Week _____

Time	Food and/or Drink	Method of Preparation	Quantity Consumed	Brand Name

Adapted From: Lee RD, Nieman DC. *Nutritional Assessment*. 2nd ed. United States of America: Mosby; 1996

INSTRUCTIONS FOR KEEPING YOUR 24-HOUR FOOD RECORD

Keep your record for three days per trial. You will include the day before, the day of, and the day after each trial. Include all meals, snacks, nibbling, and beverages including water and cocktails

1. Fill out the date and day of the week at the top of food record sheet
2. Record the time you consumed your food and/or drink. To be most accurate, fill out the food record as soon as you finish eating.
3. List the first food and/or drink you consumed when you began your day and continue to record until you consume your last food and/or drink of your day (usually before bedtime)
4. List each food and/or drink on a separate line
Example: cereal with milk, cereal and milk should each be on separate lines spaghetti, noodles, and sauce should each be on separate lines
 Combination foods:
 List parts of food on separate lines
 Include preparation method, quantity, and brand name of each food *Example: Sandwich (4 oz. healthy choice turkey, 2 slices Sara Lee wheat bread, 1 tbsp. Heilman's light mayo, 2 oz. Kraft American cheese, 1 slice of red fresh tomato)*
5. Record the method of preparation
Example: fried, baked, grilled
salt, oil (olive, canola, corn, other) butter or margarine, spices, etc.
6. Record quantity consumed
 Do not record any food not eaten
Example: made two cups of vegetables but ate half so you would record one cup Quantity of food and/or drink
Example: cups, ounces, liters, grams, each, or other unit of measure
Example: 1 cup of vegetables, 4 ounces of meat, one medium apple
7. Record brand name
Example: fast food chain name and/or package name
Example: Wendy's, Betty Crocker, Lean Cuisine, Gatorade, Thomas Bagel
8. Place any helpful food labels in manila envelope that is attached to folder

Appendix D

48-Hour Physical Activity Records

2-Day Physical Activity Records

Subject # _____

Trial # _____

Date: _____

Date	Type of Exercise Performed	Duration of Exercise (minutes)	Intensity of Exercise (use scale below)

Intensity Scale

- 6
- 7 Very, very light
- 8
- 9 Very light
- 10
- 11 Fairly light
- 12
- 13 Somewhat hard
- 14
- 15 Hard
- 16
- 17 Very hard
- 18
- 19 Very, very hard
- 20

Appendix E.

Bedtime Nutritional Shake Nutrition Label

Nutrition Facts

Serving Size 1 bottle (8 fl oz)

Amount Per Serving

Calories 350 Calories from Fat 100

	% DV*		% DV*
Total Fat 11g	17%	Sodium 220mg	9%
Saturated Fat 1g	5%	Potassium 540mg	15%
<i>Trans</i> Fat 0g		Total Carb. 50g	17%
Polyunsaturated Fat 4.5g		Dietary Fiber <1g	<4%
Monounsaturated Fat 5g		Sugars 22g	
Cholesterol 10mg	3%	Protein 13g	26%
Vitamin A 25%	• Vitamin C 60%	• Calcium 30%	
Iron 25%	• Vitamin D 80%	• Vitamin E 30%	
Vitamin K 25%	• Thiamin 25%	• Riboflavin 25%	
Niacin 25%	• Vitamin B ₆ 25%	• Folate 25%	
Vitamin B ₁₂ 25%	• Biotin 25%	• Pantothenic Acid 25%	
Phosphorus 30%	• Iodine 25%	• Magnesium 25%	
Zinc 25%	• Selenium 30%	• Copper 25%	
Manganese 60%	• Chromium 25%	• Molybdenum 60%	
Chloride 8%			

*Percent Daily Values (DV) are based on a 2,000 calorie diet.

INGREDIENTS: WATER, CORN MALTODEXTRIN, SUGAR, MILK PROTEIN CONCENTRATE, CANOLA OIL, CORN OIL, SOY PROTEIN ISOLATE, COCOA POWDER (PROCESSED WITH ALKALI); **LESS THAN 0.5% OF:** POTASSIUM CITRATE, MAGNESIUM PHOSPHATE, WHEY PROTEIN CONCENTRATE, NATURAL AND ARTIFICIAL FLAVOR, SODIUM CITRATE, SOY LECITHIN, CALCIUM PHOSPHATE, POTASSIUM CHLORIDE, CHOLINE CHLORIDE, ASCORBIC ACID, SALT, CARRAGEENAN, POTASSIUM HYDROXIDE, FERRIC PHOSPHATE, dl-ALPHA-TOCOPHERYL ACETATE, ZINC SULFATE, NIACINAMIDE, MANGANESE SULFATE, CALCIUM PANTOTHENATE, CUPRIC SULFATE, VITAMIN A PALMITATE, THIAMINE CHLORIDE HYDROCHLORIDE, PYRIDOXINE HYDROCHLORIDE, RIBOFLAVIN, CHROMIUM CHLORIDE, FOLIC ACID, BIOTIN, SODIUM MOLYBDATE, SODIUM SELENATE, POTASSIUM IODIDE, CYANOCOBALAMIN, PHYLLOQUINONE AND VITAMIN D₃. **CONTAINS MILK AND SOY INGREDIENTS.**

Abbott Nutrition, Abbott Laboratories, Columbus, Ohio 43219-3034 USA

Appendix F

Gastrointestinal Discomfort Scale

Rating of Perceived Gastrointestinal Distress

Stomach Problems

1	2	3	4	5	6	7	8	9	10
None		Slight		Moderate		Significant		Very much	Severe

GI Cramping

1	2	3	4	5	6	7	8	9	10
None		Slight		Moderate		Significant		Very much	Severe

Bloated Feeling

1	2	3	4	5	6	7	8	9	10
None		Slight		Moderate		Significant		Very much	Severe

Diarrhea

1	2	3	4	5	6	7	8	9	10
None		Slight		Moderate		Significant		Very much	Severe

Nausea

1	2	3	4	5	6	7	8	9	10
None		Slight		Moderate		Significant		Very much	Severe

Dizziness

1	2	3	4	5	6	7	8	9	10
None		Slight		Moderate		Significant		Very much	Severe

Headache

1	2	3	4	5	6	7	8	9	10
None		Slight		Moderate		Significant		Very much	Severe

Belching

1	2	3	4	5	6	7	8	9	10
None		Slight		Moderate		Significant		Very much	Severe

Vomiting

1	2	3	4	5	6	7	8	9	10
None		Slight		Moderate		Significant		Very much	Severe

Urge to Urinate or Defecate

1	2	3	4	5	6	7	8	9	10
None		Slight		Moderate		Significant		Very much	Severe

Appendix G

Borg's 6-20 Ratings of Perceived Exertion Scale

Borg Rating of Perceived Exertion

6 No exertion at all

7
8 Extremely light

9 Very light

10

11 Light

12

13 Somewhat hard

14

15 Hard (heavy)

16

17 Very hard

18

19 Extremely hard

20 Maximal exertion

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Sportscience 2017;1–4.

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